# DNA Repair Polymorphisms and Treatment Outcomes of Patients with Malignant Mesothelioma Treated with Gemcitabine-Platinum Combination Chemotherapy

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**Introduction:** Genetic polymorphisms that affect DNA repair capacity can modulate the efficacy and toxicity of cytotoxic agents. Therefore, the aim of our study was to evaluate the influence of genetic variability in DNA repair genes on treatment outcome in patients with malignant mesothelioma (MM) treated with gemcitabine-platinum combination chemotherapy.

**Methods:** In total, 109 patients with MM were genotyped for 10 polymorphisms in XRCC1, *NBN*, *RAD51*, and *XRCC3* genes. The influence of selected polymorphisms on tumor response and occurrence of treatment-related toxicity was determined by logistic regression analysis, whereas their influence on survival was estimated by Cox proportional hazards model.

**Results:** There were no associations between the investigated polymorphisms and tumor response, but we observed a significant association between *XRCC1* 399Gln allele and reduced overall survival (hazards ratio = 1.70; 95% confidence interval [CI] 1.06–2.73; $p$ = 0.028). Interaction between *XRCC1* 399Gln allele and C-reactive protein levels revealed that carriers of at least one *XRCC1* 399Gln allele with C-reactive protein levels above median had significantly shorter overall survival time compared with other patients (12.9 months versus 25.3 months, log-rank $p < 0.001$). We also observed an association between *XRCC1* 399Gln and lower frequency of leukopenia (odds ratio [OR] = 0.25; 95% CI 0.09–0.67; $p$ = 0.006), neutropenia (OR = 0.24; 95% CI 0.09–0.68; $p$ = 0.007), and thrombocytopenia (OR = 0.27; 95% CI 0.09–0.84; $p$ = 0.024). In addition, *NBN* 3474A>C, *XRCC3* -316A>G, and Thr241Met polymorphisms showed significant associations with treatment-related toxicity.

**Conclusions:** Our results support the hypothesis that DNA repair gene polymorphisms, particularly *XRCC1* Arg399Gln, may modify the response to gemcitabine-platinum combination chemotherapy and, for the first time, show this effect in patients with MM.

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Malignant mesothelioma (MM) is a rare tumor with an increasing incidence and a very poor prognosis. The 10-year average incidence in Slovenia is approximately 30 cases per year (www.slora.si, accessed on November 23, 2011) with a 1-year survival rate of 33%.[1] Recently, there have been important developments in the chemotherapy of MM, which have improved outcomes and prolonged survival of patients with MM. The pemetrexed-cisplatin combination chemotherapy has become a standard of care in MM treatment;[2] however, other similarly effective regimens, such as gemcitabine-cisplatin combination, are widely used.[3]

Gemcitabine exerts its cytotoxic effect mainly through inhibition of DNA synthesis by being incorporated into DNA and through inhibition of ribonucleotide reductase M1, resulting in a decrease of deoxyribonucleotide pools necessary for DNA synthesis. Incorporation of gemcitabine into DNA was reported to increase the stability of topoisomerase I cleavage complexes, leading to the accumulation of strand breaks.[4,5] Besides, platinum agents covalently bind to DNA, forming intrastrand DNA adducts or interstrand DNA crosslinks, which may also lead to generation of DNA strand breaks.[6] The synergistic cytotoxic effect of gemcitabine-cisplatin combination was observed in vitro[7] and it was suggested that this combination increases the accumulation of DNA strand breaks in MM cell lines.[8] These findings suggest that mechanisms involved in the repair of DNA strand breaks might play an important role in the response to gemcitabine-platinum treatment.

Single-strand breaks (SSBs) are repaired in a multistep process of the base-excision repair (BER) pathway. The central molecule of this pathway seems to be a scaffold protein x-ray repair crosscomplementing protein 1 (XRCC1), which coordinates repair of SSBs through interactions with other BER proteins.[9] Inadequate repair of SSBs because of a deficient BER mechanism can lead to more lethal double-strand breaks (DSBs).

The main mechanism involved in a high-fidelity repair of DSBs is the homologous recombination repair (HRR) pathway. The initial step is the recognition of DNA DSBs by meiotic recombination 11/RAD50/nibrin (MRE11/RAD50/NBN)

complex, followed by cleavage of 3′ ends of the DSB to form single-stranded tails, which invade the intact homologous DNA double helix. The RAD51 protein together with adaptor proteins, such as XRCC3, plays a central role in this process by facilitating initial steps of strand invasion. The 3′-single-stranded tails are extended by DNA polymerase and the resulting Holliday junctions are resolved to yield two intact DNA molecules.

A growing body of evidence has suggested that DNA repair mechanism can modulate the anticancer activity of cytotoxic agents and, therefore, genetic polymorphisms that affect DNA repair capacity might influence the efficacy and toxicity of gemcitabine-platinum combination chemotherapy in patients with MM. There are some reports regarding the influence of BER polymorphisms on treatment response to gemcitabine-platinum combination chemotherapy,[10,11] but evidence of the association between HRR polymorphisms and treatment outcome is insufficient. Moreover, the influence of BER or HRR polymorphisms on treatment outcome in MM patients treated with gemcitabine-platinum combination chemotherapy has not been established so far.

The aim of our study was to evaluate the influence of single-nucleotide polymorphisms (SNPs) in *XRCC1*, *NBN*, *RAD51*, and *XRCC3* genes and their corresponding haplotypes on tumor response, survival, and treatment-related toxicity in patients with MM treated with gemcitabine-platinum combination chemotherapy.

## PATIENTS AND METHODS

### Study Design

Separate case-control studies were designed for the analysis of tumor response and toxicity. For analysis of tumor response, cases were defined as patients with stable disease (SD) or progressed disease (PD), whereas controls were defined as patients with complete response (CR) or partial responses (PR) to treatment. For toxicity analyses, cases were defined as patients who developed specific treatment-related toxicities, whereas controls were defined as patients who did not develop that toxicity. For survival analysis, a Cox model was used.

### Patients

The study group consisted of 109 patients with histologically confirmed MM. All the patients were diagnosed between 1997 and 2010 at the University Clinic of Pulmonary and Allergic Diseases in Golnik, Slovenia and at the University Clinical Centre Maribor, Slovenia. The inclusion criteria for the selection of patients and details of clinical data collection were described previously.[12]

All the patients who were alive at the time of data collection gave their written informed consent to participate in the study. The study was approved by the Slovenian Ethics Committee for Research in Medicine (approval ref. no. 04/02/09) and was carried out according to the Declaration of Helsinki.

### Treatment

All patients with MM were treated at the Institute of Oncology, Ljubljana, Slovenia; therefore, treatment, outcome assessment, and follow-up were centralized for all subjects. Patients were treated with gemcitabine in combination with a platinum agent according to one of the two following regimens: gemcitabine in prolonged infusion in combination with cisplatin or carboplatin;[13] or gemcitabine in standard infusion in combination with cisplatin.[14] We also included patients who received gemcitabine-platinum combination chemotherapy as a part of multimodality treatment with surgery and/or palliative radiotherapy.

### Response, Survival, and Toxicity Assessment

Tumor response was evaluated as described previously.[14] Progression-free survival (PFS) time was defined as time from day 1 of first-line gemcitabine-platinum chemotherapy to the day of documented disease progression according to the Response Evaluation Criteria In Solid Tumors or to death from any cause, whichever occurred first. Overall survival (OS) time was defined as time from day 1 of first-line gemcitabine-platinum chemotherapy to death from any cause. Patients without documented progression or death at the last follow-up evaluation (September 2011) were censored at that time. Hematologic toxicities, nephrotoxicity, alopecia, and nausea/vomiting were evaluated according to the National Cancer Institute-Common Toxicity Criteria, version 4.0 (http://ctep.info.nih.gov/reporting/ctc.html, accessed on November 23, 2011). Hematologic toxicities were defined by decreased serum hemoglobin levels (anemia), decline of: white blood cells (leukopenia), neutrophil (neutropenia), and platelet count (thrombocytopenia). Nephrotoxicity was defined by elevated levels of serum creatinine concentration. The highest grade of individual toxicity during first-line chemotherapy was chosen as the endpoint for toxicity analyses. Toxicities of grade 2 or higher were considered as clinically relevant. Thrombocytopenia and nephrotoxicity was categorized only as present or absent because of the very low frequency of grade 2 or higher toxicities in the study group.

### SNP Selection

SNP search in *XRCC1*, *NBN*, *RAD51*, and *XRCC3* genes was assessed using the scientific literature database PubMed (http://www.ncbi.nlm.nih.gov/pubmed), National Center for Biotechnology Information SNP database,[15] and HapMap database.[16] Previously investigated functional SNPs and/or putatively functional SNPs that tag haplotype blocks with minor allele frequencies greater than 5% were selected. The exploration of possible SNPs' functionality and haplotype-tagging was carried out by Web-based SNP prediction tools.[17,18]

### DNA Extraction and Genotyping

Tumor tissue specimens or peripheral blood samples were collected at the time of diagnosis. Tumor tissue specimens were routinely formalin fixed and paraffin embedded. Genomic DNA from formalin-fixed and paraffin-embedded tissue was extracted as previously described.[19] A Qiagen FlexiGene kit (Qiagen, Hilden, Germany) was used for the extraction of genomic DNA from frozen whole-blood samples.

Genotypes of *XRCC1* were determined by TaqMan SNP genotyping method (Applied Biosystems, Foster City, CA),

as described previously.[20] Genotyping of *NBN*, *RAD51*, and *XRCC3* was carried out using a fluorescence-based competitive allele-specific (KASPar) assay (KBiosciences, Herts, United Kingdom).[21] To ensure the data quality, 10% of the samples were genotyped in duplicates, and samples with discordant results were excluded from the data analysis.

## Statistical Analysis

The median was used to present central tendency, whereas the range (minimum–maximum) was a measure of variability. For each SNP, deviations of genotype frequency distribution between blood and tumor samples, as well as deviations of genotype frequency distribution from those expected under Hardy-Weinberg equilibrium (HWE) were assessed using the standard $\chi^2$ test.

A dominant genetic model was used in all statistical analyses. The number of different treatment-related toxicities in individual patients was compared between genotype groups using the nonparametric Mann-Whitney *U* test. The influences of investigated SNPs on tumor response and occurrence of treatment-related toxicities were examined by logistic regression analysis to calculate odds ratios (ORs) and their respective 95% confidence intervals (CIs).

Survival times were calculated and compared using the Kaplan–Meier method. Survival curves were estimated using the Cox proportional hazards model, and hazard ratios (HRs) with their 95% CIs were determined. All potential clinical and treatment predictors[12] were independently analyzed for their influence on tumor response, PFS, OS, and treatment-related toxicity. For predictors with *p* values less than 0.050 in the univariable analysis forward selection was applied, and only significant predictors were included in the final multivariable model.

Stratification was performed to investigate possible confounding or effect modification of different variables. In case of observed effect modification, the regression model using dummy variables was introduced. All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) for Windows, version 19 (IBM Corporation, Armonk, NY).

On the basis of genotype data, haplotypes were reconstructed and analyzed using the Thesias program,[22] as described previously.[14] The influence of investigated haplotypes on tumor response, OS, PFS, and treatment-related toxicity was assessed. All statistical tests were two sided and the level of significance for all statistical analyses was set to 0.050.

# RESULTS

## Patients

The study included 109 patients with either pleural ($n = 99$) or peritoneal ($n = 10$) MM. Demographic and clinical characteristics of the study group are summarized in Table 1. The study group consisted of 83 men (76.1%) and 26 women (23.9%) with a median age of 62 years (range, 32–83 years) at the time of diagnosis (Table 1). All patients received gemcitabine in combination with platinum agents during the first-line setting. Most of these patients ($n = 105$, 96.3%) were treated with gemcitabine in prolonged infusion in combination with a platinum agent, and 81 patients (74.3%) received six cycles of first-line chemotherapy. Eighteen patients (16.5%) underwent multimodality treatment. Forty-eight patients (43.6%) received second-line chemotherapy and, among them, 17 (35.4%) received six cycles of chemotherapy. As a general rule, patients who previously responded to treatment with gemcitabine in prolonged infusion in combination with a platinum agent were considered for reinduction with the same or similar chemotherapy schedule ($n = 30$). Other treatment options included combination of pemetrexed with platinum agent ($n = 12$), navelbine ($n = 6$), or palliative radiotherapy ($n = 14$).

Patients who underwent surgical resection of tumor before chemotherapy ($n = 4$, 3.7%) were not evaluated in the tumor response analysis. Among 105 assessable patients for tumor response analysis, there were five patients (4.8%) with CR and 47 (44.8%) with PR, together accounting for an overall response rate of 49.6%. SD was observed in 47 patients (44.8%) and six patients had progressed disease (5.7%). At

**TABLE 1.** Demographic and Clinical Characteristics of Patients with Malignant Mesothelioma Treated with Gemcitabine-Platinum Combination Chemotherapy (N = 109)

| Characteristic | n (%) | Median (Range) |
|---|---|---|
| **Age, yrs** | | 62 (32–83) |
| **Sex** | | |
| Male | 83 (76.1) | |
| Female | 26 (23.9) | |
| **Asbestos exposure**[a] | 78 (71.6) | |
| **Smoking status**[b] | | |
| Smoker | 47 (43.1) | |
| Nonsmoker | 60 (55.0) | |
| **ECOG performance status** | | |
| <2 | 76 (69.7) | |
| ≥2 | 33 (30.3) | |
| **Histological type** | | |
| Epitheloid | 79 (72.4) | |
| Biphasic | 17 (15.6) | |
| Sarcomatoid | 10 (9.2) | |
| Not characterized | 3 (2.8) | |
| **TNM stage** | | |
| I or II | 36 (33.0) | |
| III or IV | 63 (57.8) | |
| Not characterized | 10 (9.2) | |
| **Percentage of weight loss, %**[c] | | 2 (0-22) |
| **Pain at diagnosis**[c] | 74 (67.9) | |
| **Hemoglobin level at diagnosis, g/L**[d] | | 131 (88–167) |
| **White blood cell count at diagnosis, *10$^9$/L**[d] | | 8.6 (3.1-19.8) |
| **LDH level at diagnosis, µkat/L** | | 2.8 (1.5-109.0) |
| **CRP level at diagnosis, mg/L** | | 26 (0-339) |

[a]Asbestos exposure was defined as occupational or environmental exposure.[12]
[b]Data missing for two (1.8%) patients.
[c]Data missing for three (2.8%) patients.
[d]Data missing for one (0.9%) patient.
CRP, C-reactive protein; ECOG, Eastern Cooperative Oncology group; LDH, lactate dehydrogenase; TNM, tumor-nodes-metastasis.

 

**TABLE 2.** Influence of BER and HRR Polymorphisms on Tumor Response and Survival Assessed in Univariable Analyses in Patients with Malignant Mesothelioma Treated with Gemcitabine-Platinum Combination Chemotherapy (N = 109)

| | | | Patients | Tumor response[a] | | PFS | | OS | |
|---|---|---|---|---|---|---|---|---|---|
| **Polymorphism** | **Genotype** | ***n*** | **(%)** | **OR (95% CI)[b]** | ***p*** | **HR (95% CI)[c]** | ***p*** | **HR (95% CI)[c]** | ***p*** |
| ***XRCC1* Arg194Trp rs1799782** | Arg/Arg | 91 | (83.5) | 1 | | 1 | | 1 | |
| | Arg/Trp | 18 | (16.5) | 0.73 (0.25–2.13) | 0.560 | 0.78 (0.45–1.34) | 0.372 | 0.79 (0.46–1.36) | 0.394 |
| | Trp/Trp | 0 | (0.0) | | | | | | |
| ***XRCC1* Arg399Gln rs25487** | Arg/Arg | 39 | (35.8) | 1 | | 1 | | 1 | |
| | Arg/Gln | 58 | (53.2) | 0.68 (0.30–1.52) | 0.343 | 1.11 (0.73–1.67) | 0.637 | 1.18 (0.77–1.82) | 0.443 |
| | Gln/Gln | 12 | (11.0) | | | | | | |
| ***NBN* Glu185Gln rs1805794** | Glu/Glu | 17 | (15.6) | 1 | | 1 | | 1 | |
| | Glu/Gln | 44 | (40.4) | 1.56 (0.55–4.55) | 0.405 | 0.73 (0.43–1.26) | 0.257 | 0.76 (0.43–1.35) | 0.350 |
| | Gln/Gln | 48 | (44.0) | | | | | | |
| ***NBN* 1197A>G rs709816** | A/A | 45 | (41.3) | 1 | | 1 | | 1 | |
| | A/G | 46 | (42.2) | 0.95 (0.44–2.08) | 0.907 | 1.08 (0.73–1.62) | 0.694 | 0.90 (0.60–1.36) | 0.624 |
| | G/G | 18 | (16.5) | | | | | | |
| ***NBN* 3474A>C rs1063054** | A/A | 47 | (43.1) | 1 | | 1 | | 1 | |
| | A/C | 45 | (41.3) | 0.95 (0.48–2.08) | 0.910 | 1.13 (0.75–1.68) | 0.565 | 0.91 (0.60–1.38) | 0.657 |
| | C/C | 17 | (15.6) | | | | | | |
| ***RAD51* -98G>C rs1801320** | G/G | 90 | (82.6) | 1 | | 1 | | 1 | |
| | G/C | 17 | (15.6) | 2.50 (0.87–7.14) | 0.090 | 0.88 (0.53–1.48) | 0.636 | 0.85 (0.48–1.51) | 0.583 |
| | C/C | 2 | (1.8) | | | | | | |
| ***RAD51* 1522T>G rs12593359** | T/T | 24 | (22.0) | 1 | | 1 | | 1 | |
| | T/G | 55 | (50.5) | 1.96 (0.78–5.00) | 0.152 | 0.98 (0.61–1.56) | 0.920 | 1.16 (0.71–1.89) | 0.560 |
| | G/G | 30 | (27.5) | | | | | | |
| ***XRCC3* -316A>G rs1799794** | A/A | 61 | (56.3) | 1 | | 1 | | 1 | |
| | A/G | 44 | (40.4) | 1.04 (0.48–2.27) | 0.910 | 1.18 (0.81–1.74) | 0.413 | 1.14 (0.75–1.72) | 0.545 |
| | G/G | 4 | (3.6) | | | | | | |
| ***XRCC3* Thr214Met rs861539** | Thr/Thr | 43 | (39.4) | 1 | | 1 | | 1 | |
| | Thr/Met | 43 | (39.4) | 0.64 (0.29–1.41) | 0.266 | 1.15 (0.77–1.72) | 0.493 | 1.09 (0.71–1.67) | 0.690 |
| | Met/Met | 23 | (21.1) | | | | | | |

[a]Complete or partial response versus stable or progressed disease. Data on tumor response was available for 105 (96.3%) patients.
[b]ORs, 95% CIs, and *P* values were calculated by univariable logistic regression and the dominant genetic model was used.
[c]HRs, 95% CIs, and *P* values were calculated by univariable Cox proportional hazards regression and the dominant genetic model was used.
BER, base-excision repair; CI, confidence interval; HR, hazard ratio; HRR, homologous recombination repair; OR, odds ratio; OS, overall survival; PFS, progression-free survival.

the date of the last follow-up, 102 patients (93.6%) had experienced disease progression and 93 had died (85.3%). Median PFS time was 8 months (range, 0–63 months), whereas OS time was 16 months (range, 1–85 months). Median follow-up time for living patients was 16 months (range, 4–85 months).

One hundred patients (91.8%) developed at least one toxic effect and the number of different treatment-related toxicities in individual patients ranged from one to six. Prevalence of treatment-related toxicities was as follows: anemia of grade 2 or higher ($n$ = 61; 56.0%), leukopenia grade of 2 or higher ($n$ = 43; 39.4%), neutropenia of grade 2 or higher ($n$ = 57; 52.3%), thrombocytopenia of grade 1 or higher ($n$ = 17; 15.6%), alopecia of grade 2 or higher ($n$ = 63; 57.8%), and nausea/vomiting of grade 2 or higher ($n$ = 44; 40.4%).

## Genotyping Analysis

According to the SNP selection criteria, 10 SNPs in *XRCC1*, *NBN*, *RAD51*, and *XRCC3* genes were selected, and their genotype frequencies in patients with MM are presented in Table 2. All genotype frequencies were consistent with HWE and with frequencies reported for other white population in National Center for Biotechnology Information SNP database, except for *RAD51* -61G>T SNP, which showed significant deviation from HWE ($p$ = 0.016) as well as from published genotype frequencies in the white population ($p$ = 0.023). Therefore, *RAD51–61*G>T SNP was excluded from further statistical analysis.

Because DNA samples of patients with MM were obtained either from tumor samples ($n$ = 42, 38.5%) or peripheral blood lymphocytes ($n$ = 67, 61.5%), both DNA sources were tested for possible genotype discrepancies. In 10 patients who had both DNA sources available, genotypes of all investigated SNPs were in complete concordance between germline and tumor DNA. Moreover, we observed no statistically significant differences of genotype-frequency distributions between tumor and blood samples or deviations from HWE in the group of tumor samples (see Table, Supplemental Digital

Content 1, http://links.lww.com/JTO/A321, which shows comparison of distributions of genotype frequencies between blood and tumor samples and deviations from HWE in the group of tumor samples), indicating no loss of heterozygosity.

## Tumor Response Analysis

The influence of *XRCC1*, *NBN*, *RAD51*, and *XRCC3* SNPs on tumor response was determined by univariable logistic regression analysis. No significant associations were found between investigated SNPs and patients who achieved CR or PR (Table 2).

## Survival Analysis

The influence of *XRCC1*, *NBN*, *RAD51*, and *XRCC3* SNPs on PFS and OS was determined by univariable Cox proportional hazards model, but no significant associations between investigated SNPs and survival were observed (Table 2). Among the clinical variables with evidence of prognostic impact, sarcomatoid histological type (HR = 2.46; 95% CI 1.16–5.21; $p = 0.019$), C-reactive protein (CRP) level at the time of diagnosis (HR = 1.01; 95% CI 1.00–1.01; $p < 0.001$), number of first-line chemotherapy cycles (HR = 0.74; 95% CI 0.61–0.90; $p = 0.002$), and number of second-line chemotherapy cycles (HR = 0.86; 95% CI 0.78–0.95; $p = 0.003$) remained significant predictors of OS in multivariable model. When this model was used, *XRCC1* Arg399Gln SNP showed a statistically significant influence on OS (HR = 1.70; 95% CI 1.06–2.73; $p = 0.028$).

Sarcomatoid histological type and number of second-line chemotherapy cycles did not substantially influence hazard of dying for *XRCC1* 339 Arg/Arg versus Arg/Gln+Gln/Gln genotypes, but the adjustment for CRP level at the time of diagnosis and number of first-line chemotherapy cycles changed HR from 1.18 (95% CI 0.77–1.82) in univariable analysis to 1.40 (95% CI 0.91–2.18) and 1.51 (95% CI 0.95–2.39), respectively (see Table, Supplemental Digital Content 2, http://links.lww.com/JTO/A322, which demonstrates changes in HR for *XRCC1* Arg399Gln SNP after adjustment for clinical predictors). Stratification of association between *XRCC1* Arg399Gln SNP and OS by CRP level at the time of diagnosis and number of first-line chemotherapy cycles suggested a possible interaction between *XRCC1* Arg399Gln SNP and CRP level at the time of diagnosis (see Tables, Supplemental Digital Content 3 and 4, http://links.lww.com/JTO/A323 and http://links.lww.com/JTO/A324, which demonstrate stratification of association between *XRCC1* Arg399Gln SNP and OS by CRP level at the time of diagnosis and number of first-line chemotherapy cycles). Using Cox proportional hazards model, a significant interaction between *XRCC1* Arg399Gln SNP and CRP level at diagnosis as a dichotomous variable was observed (HR = 2.52; 95% CI 1.04–6.08; $p = 0.040$) (Table 3).

Carriers of at least one *XRCC1* 399Gln allele, who had above-median CRP levels ($n = 35$, 32.1%) relative to other patients had significantly shorter median OS time (10.0 months versus 17.0 months, log-rank $p < 0.001$) (Fig. 1) and significantly worse OS probability in the multivariable model adjusted for sarcomatoid histological type, number of first-line chemotherapy cycles, and number of second-line chemotherapy cycles (HR = 2.47; 95% CI 1.56–3.92; $p < 0.001$) (Fig. 1). Carriers of at least one *XRCC1* 399Gln allele, who had above-median CRP levels received similar chemotherapy combination regimens in the second-line setting compared with other patients ($p = 0.297$). Besides, we did not observe any influence of the type of second-line chemotherapy regimen on OS (log-rank $p = 0.462$).

**TABLE 3.** Influence of Interaction between XRCC1 Arg399Gln Polymorphism and CRP Levels at Diagnosis as Dichotomous Variable on Overall Survival in Cox Proportional Hazards Model in Patients with Malignant Mesothelioma Treated with Gemcitabine-Platinum Combination Chemotherapy (N = 109)

| Variable | HR (95% CI) | *p* |
|---|---|---|
| ***XRCC1* 399Arg/Arg versus 399Arg/Gln+Gln/Gln** | 0.81 (0.46–1.45) | 0.485 |
| **CRP level <median versus ≥median** | 0.95 (0.47–1.94) | 0.897 |
| ***XRCC1* Arg399Gln*CRP level** | 2.52 (1.04–6.08) | 0.040 |

HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein.

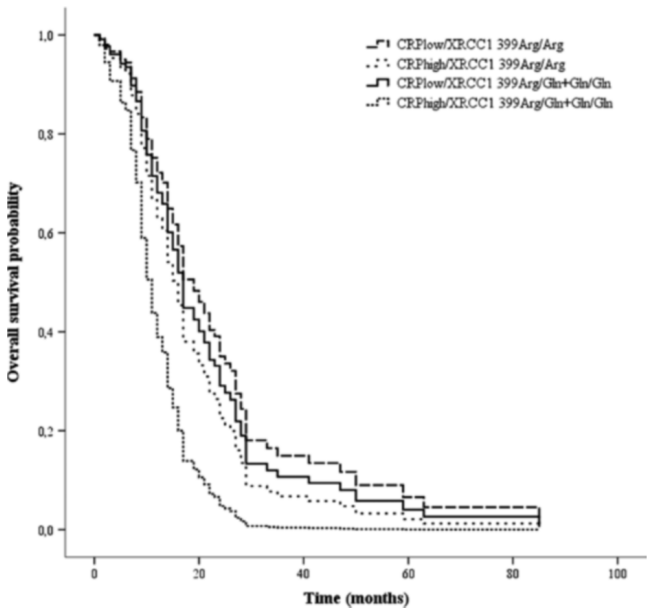


**FIGURE 1.** Overall survival in the function of combination of CRP levels and *XRCC1* Arg399Gln genotype in malignant mesothelioma patients treated with gemcitabine-platinum combination chemotherapy (N = 109). CRP, C-reactive protein; CRPhigh, above-median CRP level; CRPlow, under-median CRP level.

## Toxicity Analysis

In univariable logistic regression analysis only *XRCC1* Arg399Gln, *NBN* 3474A>C, *XRCC3* -316A>G, and *XRCC3* Thr241Met polymorphisms influenced treatment-related toxicity (Table 4). None of the investigated polymorphisms were associated with anemia of grade 2 or higher or nephrotoxicity of grade 1 or higher ($p \geq 0.050$ for all associations).

 

**TABLE 4.** Influence of BER and HRR Polymorphisms on Occurrence of Treatment-Related Toxicities Assessed in Univariable Analyses in Patient with Malignant Mesothelioma Treated with Gemcitabine-Platinum Combination Chemotherapy (N = 109)

| | Leukopenia[a] grade ≥2 | | Neutropenia grade ≥2 | | Thrombocytopenia[a] grade ≥2 | | Alopecia[b] grade ≥2 | | Nausea/vomiting[b] grade ≥2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Polymorphism** | **OR (95% CI)** | ***p*** | **OR (95% CI)** | ***p*** | **OR (95% CI)** | ***p*** | **OR (95% CI)** | ***p*** | **OR (95% CI)** | ***p*** |
| ***XRCC1 Arg399Gln*** | | | | | | | | | | |
| Arg/Arg versus Arg/Gln+Gln/Gln | **0.40 (0.18–0.90)** | **0.027** | **0.40 (0.18–0.90)** | **0.027** | **0.33 (0.11–0.95)** | **0.039** | 0.90 (0.40–2.02) | 0.797 | 0.48 (0.21–1.08) | 0.075 |
| ***NBN* 3474A>C** | | | | | | | | | | |
| A/A versus A/C+C/C | 1.70 (0.77–3.77) | 0.189 | **2.33 (1.08–5.06)** | **0.032** | 1.97 (0.64–6.04) | 0.237 | **2.63 (1.19–5.82)** | **0.017** | 1.36 (0.62–2.97) | 0.448 |
| ***XRCC3* -316A>G** | | | | | | | | | | |
| A/A versus A/G+G/G | 1.15 (0.53–2.49) | 0.725 | 0.85 (0.40–1.81) | 0.617 | **0.22 (0.06–0.81)** | **0.023** | 1.81 (0.82–3.97) | 0.141 | 1.95 (0.89–4.26) | 0.094 |
| ***XRCC3* Thr241Met** | | | | | | | | | | |
| Thr/Thr versus Thr/Met+Met/Met | 0.81 (0.37–1.79) | 0.607 | 1.26 (0.58–2.71) | 0.560 | 1.20 (0.41–3.53) | 0.741 | 0.81 (0.37–1.80) | 0.609 | **0.34 (0.18–0.88)** | **0.023** |

ORs, 95% CIs, and *p* values were calculated by univariable logistic regression. Bold characters indicate statistically significant results.
[a]Toxicity data missing for one (0.9%) patient.
[b]Toxicity data missing for two (1.8%) patients.
BER, base-excision repair; CI, confidence interval; HRR, homologous recombination repair; OR, odds ratio.

Among the investigated clinical parameters, female sex, age, nonsarcomatoid histology, and higher number of first-line chemotherapy cycles were identified as independent predictors of different treatment-related toxicities in the multivariable model (Table 5). When this model was used, *XRCC1* 399Arg/Gln+Gln/Gln genotypes remained significantly but negatively associated with leukopenia of grade 2 or higher (OR = 0.25; 95% CI 0.09–0.67; $p = 0.006$), neutropenia of grade 2 or higher (OR = 0.24; 95% CI 0.09–0.68; $p = 0.007$), and thrombocytopenia of grade 1 or higher (OR = 0.27; 95% CI 0.09–0.84; $p = 0.024$). In addition, the following association of HRR SNPs with treatment-related toxicity remained significant in the multivariable model: *NBN* 3474A/C+C/C genotypes with increased odds ratio of alopecia of grade 2 or higher (OR = 2.81; 95% CI 1.13–7.00; $p = 0.027$), *XRCC3* -316A/G+G/G genotypes with odds ratio below one of thrombocytopenia of grade 1 or higher (OR = 0.18; 95% CI 0.05–0.68; $p = 0.012$), and *XRCC3* 241Thr/Met+Met/Met genotypes with odds below one of nausea/vomiting of grade 2 or higher (OR = 0.34; 95% CI 0.14–0.85; $p = 0.021$).

We also determined the influence of investigated polymorphisms on the number of different treatment-related toxicities in individual patients. A significantly lower number of different toxicities was observed in carriers of at least one *XRCC1* 399Gln compared with patients with wild-type genotype (Kendal $\tau = -0.192$, $p = 0.026$), whereas the number of different toxicities was higher in carriers of at least one *NBN* 3474C allele compared with patients with wild-type genotype (Kendal $\tau = 0.228$, $p = 0.008$).

**TABLE 5.** Influence of Clinical and Treatment Characteristics on the Occurrence of Treatment-Related Toxicity Assessed in Multivariable Analysis in Patients with Malignant Mesothelioma Treated with Gemcitabine-Platinum Combination Chemotherapy (N = 109)

| Treatment-Related toxicity | Characteristic | OR (95% CI) | *p* |
|---|---|---|---|
| Leukopenia grade ≥2[a] | Female sex | 3.45 (1.13–10.53) | 0.030 |
| | Number of first-line | 2.10 (1.12–3.94) | 0.021 |
| Neutropenia grade ≥2 | Sarcomatoid histology | 0.11 (0.01–0.92) | 0.042 |
| | Number of first-line chemotherapy cycles | 1.58 (1.03–2.42) | 0.035 |
| Alopecia grade ≥2[b] | Sarcomatoid histology | 0.15 (0.03–0.79) | 0.025 |
| | Number of first-line chemotherapy cycles | 1.52 (1.02–2.27) | 0.042 |
| Nausea/vomiting grade ≥2[b] | Female sex | 3.24 (1.08–9.70) | 0.035 |
| | Age | 0.93 (0.88–0.98) | 0.004 |

ORs, 95% CIs, and *p* values were calculated by multivariable logistic regression model including sex, age, histological type, and number of first-line chemotherapy cycles.
[a]Toxicity data missing for one (0.9%) patient.
[b]Toxicity data missing for two (1.8%) patients.
CI, confidence interval; OR, odds ratio.

## Haplotype Analysis

Haplotype analysis was carried out to evaluate the combined effect of BER and HRR SNPs on treatment outcome in patients with MM. Three 2-SNP *XRCC1* (Arg194Trp, Arg399Gln) haplotypes, three 3-SNP *NBN* (Glu185Gln, 1197A>G, 3474A>C) haplotypes, three 2-SNP *RAD51* (-98G>C, 1522T>G) haplotypes, and three 2-SNP *XRCC3* (-316A>G, Thr241Met) haplotypes had a frequency greater than 5% (see Table, Supplemental Digital Content 5, http://links.lww.com/JTO/A325, which shows haplotype frequencies). There were no significant associations between the investigated haplotypes and tumor response, PFS, or OS (data not shown). Nevertheless, carriers of *XRCC1* CA haplotype had significantly lower odds ratio of leukopenia of grade 2 or higher (OR = 0.53; 95% CI 0.29–0.98; $p = 0.044$) and thrombocytopenia of grade 1 or higher (OR = 0.39; 95% CI

0.16–0.95; $p = 0.037$) compared with patients with the wild-type *XRCC1* CG haplotype. We also observed an association between *XRCC3* GC haplotype and lower frequency of thrombocytopenia of grade 1 or higher compared with *XRCC3* AT haplotype (OR = 0.13; 95% CI 0.02–0.75; $p = 0.022$).

## DISCUSSION

In this study, we evaluated the effects of ten SNPs in BER and HRR pathway on treatment outcome in patients with MM treated with gemcitabine-platinum combination chemotherapy. None of the investigated polymorphisms influenced tumor response or PFS. We also observed a highly significant decrease of OS probability in carriers of *XRCC1* 399Gln allele, who had above-median CRP levels at the time of diagnosis. Our results are in agreement with the current understanding of *XRCC1* involvement in platinum-based chemotherapy. Several studies, which investigated the influence of *XRCC1* SNPs on treatment outcome in cancer patients treated with different platinum-based chemotherapy regimens, showed a significant association of *XRCC1* 399Gln allele with worse clinical outcomes.[23–27] Among five studies published to date, which included gemcitabine-platinum treated NSCLC patients, three of them reported shorter median OS time[11,28] or lower response rates in carriers of *XRCC1* 399Gln allele.[11,29] On the contrary, Giachino et al.[30] reported a borderline significant opposing effect of *XRCC1* 399Gln variant on OS, whereas de las Peñas et al.[10] showed a favorable prognosis of patients with heterozygous genotype relative to patients with both homozygous genotypes. However, none of these studies investigated interactions between *XRCC1* Arg399Gln polymorphism and clinical prognostic factors.

It was suggested that XRCC1 protein is involved in both BER and SSB repair pathways, and may therefore play a role in the response to gemcitabine-platinum combination therapy. Because *XRCC1* Arg399Gln SNP is located in the poly-(ADP-ribose) polymerase 1-binding domain, it was suggested that this SNP affects the protein's function. Its functional significance was shown by cytogenetic challenge assay, indicating a defective BER function in X-irradiated cells homozygous for the *XRCC1* 399Gln variant.[31] These observations were also confirmed in vivo, as carriers of *XRCC1* 399Gln variant allele were reported to have higher levels of DNA damage compared with wild-type individuals.[32,33] These reports, together with our findings, support the hypothesis that DNA repair polymorphisms, contributing to suboptimal DNA repair capacity, result in more biologically aggressive tumors and promote cancer progression rather than influence response to genotoxic agents through inefficient repair.[34,35] This assumption was further confirmed by the interaction between *XRCC1* Arg399Gln SNP and CRP levels influencing OS probability in our study. Decreased DNA repair capacity, caused by the *XRCC1* 399Gln variant, and high CRP levels might be both associated with more aggressive tumors;[35,36] therefore, a highly significant decrease of OS probability in patients with *XRCC1* 399Gln allele, who had high CRP levels at diagnosis, was expected. Nevertheless, CRP level at diagnosis and *XRCC1* Arg399Gln polymorphisms by themselves did not have a statistically significant impact on survival in multivariate analysis including interaction parameter. Because of wide confidence intervals which included one, these findings are preliminary and need to be validated in larger cohorts of similarly treated patients.

Toxicity analyses carried out in our study showed significantly decreased odds of leukopenia, neutropenia, and thrombocytopenia as well as significantly lower numbers of different treatment-related toxicities in carriers of polymorphic *XRCC1* 399Gln allele. As found in single SNP analysis, carriers of the *XRCC1* CA haplotype, containing polymorphic 399Gln allele, had fewer leukopenia and thrombocytopenia events than patients with wild-type CG haplotype. In concordance with our results, other studies also reported an association between decreased susceptibility to treatment-related toxicity and *XRCC1* 399Gln allele.[37–39] Nevertheless, these associations are not in agreement with the functional significance of *XRCC1* Arg399Gln SNP, as decreased DNA repair capacity is expected to result in a higher frequency of toxic effects in normal tissues. A possible explanation of these discrepant findings is that *XRCC1* Arg399Gln SNP is not the casual variant, but is rather in linkage disequilibrium with another functional polymorphism. A study supporting this assumption showed a significant association of *XRCC1* Arg399Gln SNP with protein expression in advanced cervical carcinoma patients.[23]

Moreover, polymorphisms of HRR pathway also showed significant associations with treatment-related toxicities in patients with MM treated with gemcitabine-platinum combination chemotherapy. We observed significantly higher odds of neutropenia and alopecia as well as higher number of different treatment-related toxicities in carriers of *NBN* 4374C allele relative to patients with the wild-type genotype. To date, *NBN* 4374A<C SNP was shown to increase susceptibility to lung cancer,[40] but its association with clinical outcome has not been determined yet. Because the functional significance of *NBN* 4374A<C SNP is also unknown, the biologic interpretation of this data is difficult. This SNP is located in the 3′ untranslated region of *NBN* gene and several micro-RNA–binding sites were predicted in this region,[17] suggesting a possible influence of 4374A<C SNP on the translational efficacy of the *NBN* gene. Therefore, further investigations are needed to confirm the clinical relevance and the biological function of this SNP.

Our study showed no association of *RAD51* -98G>C and 1522T>G SNPs with clinical outcome in patients with MM, whereas -61G>T SNP showed a significant deviation from HWE, and was thus excluded from statistical analyses. In contrast to our results, *RAD51* -98G>C polymorphism showed a significant prognostic role in NSCLC patients treated with a platinum agent in combination with gemcitabine and taxanes,[41] but another study reported no association of this polymorphism with treatment outcome in platinum-based treated breast cancer patients.[42] The lack of association between *RAD51* -98G>C SNP and treatment outcomes in our study might be because of the low frequency of the polymorphic allele and a relatively small study population, leading to insufficient statistical power to detect significant associations.

Both SNPs, investigated in the *XRCC3* gene, influenced treatment-related toxicity in our group of patients with MM. We observed significant associations between polymorphic

 

*XRCC3* -316G allele and decreased odds of thrombocytopenia, as well as between polymorphic *XRCC3* 241Met allele and decreased odds of nausea/vomiting. The influence of *XRCC3* SNPs on treatment-related toxicity was confirmed in haplotype analysis and demonstrated a significant association between *XRCC3* GC haplotype and increased frequency of thrombocytopenia. Similar results were obtained in other studies, where *XRCC3* 241Met allele showed protective effects toward radiation-induced toxicity in normal tissue[43] and liver toxicity in acute myeloid leukemia patients.[44] The only study investigating the influence of *XRCC3* -316A>G SNP on treatment-related toxicity reported that the *XRCC3* -316G allele was significantly associated with higher level of radiation-induced toxicity, but this association did not remain significant in the multivariable model.[45]

The analysis of potential clinical predictive and prognostic factors in this study showed an important role for histological type, CRP level at diagnosis, and number of first-line and second-line chemotherapy cycles in the survival of patients with MM. Compared with our previous study, the significance of the association between CRP level and OS was even higher in this cohort of patients with updated survival data, indicating a potential clinical importance of CRP levels at diagnosis as a prognostic marker.[46,47] Our favorable experience and a recent report on reinduction of pemetrexed-based second-line chemotherapy indicated that at least some patients did not develop resistance to the prior chemotherapy combination.[13,48] However, we did not observe the difference in OS between patients who received gemcitabine-based or pemetrexed-based combination chemotherapy in the second-line setting. In addition, we identified female sex, younger age, histological type, and number of first-line chemotherapy cycles as predictors of treatment-related toxicity. As gemcitabine pharmacokinetics was shown to be significantly influenced by sex,[49] the increased frequency of treatment-related toxicity among women was expected. Similar to our findings, the decreased frequency of chemotherapy-induced nausea/vomiting in older patients was reported in another study.[50] However, the association between histological type and treatment-related toxicity has not been investigated yet.

Despite several significant findings reported in this study, some potential limitations should be taken into consideration. We are aware that in some cases low statistical power, caused by a relatively small sample size, might lead to false findings. However, several highly significant associations with very narrow confidence intervals were observed, indicating a low probability of false-positive findings. To assess the possible confounding effects of the various clinical parameters, all variables that might have affected treatment outcomes were examined in the multivariable analyses. In addition, the use of different DNA sources for genotyping analysis might have influenced our results but, similar to our previous studies,[12,14] we confirmed a strong concordance between different DNA sample sources at the single-nucleotide level. Our observations were also in good agreement with studies that showed strong concordance between different DNA sample sources at the single-nucleotide level.[51]

The major strength of our study was a relatively homogenous patient cohort because only patients with MM treated with first-line gemcitabine-platinum combination chemotherapy were included. As treatment, outcome assessment, and follow-up were centralized for all included patients with MM, discrepancies in the clinical data collection procedure were minimized. Besides, our study was not biased by genetic heterogeneity because all the patients were recruited from a geographically limited area with an ethnically homogeneous population.[52]

In conclusion, our results support the hypothesis that DNA repair gene polymorphisms, particularly *XRCC1* Arg399Gln, may modulate the response to gemcitabine-platinum combination chemotherapy and, for the first time, show this effect in patients with MM. Although these findings are of interest, they should be replicated in independent prospective studies to validate the importance of BER and HRR polymorphisms in gemcitabine-platinum combination chemotherapy treatment outcomes. Because response to any systemic chemotherapy in patients with MM is generally low, individualized chemotherapy strategies that allow selection of patients who would most likely benefit from gemcitabine-platinum treatment should be considered.

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